

Geological Factors and Biochemical Aspects of the Origin of Land Plants

Introduction

In this chapter I will consider a possible relationship between the land migration and (1) the development of an ultraviolet (UV) radiation screen and (2) the development of an atmosphere with enough oxygen to produce this screen and to provide for the biosynthesis of cutin, the plant waterproofing agent. Both developments may be correlated with an increasing partial pressure of oxygen. The discussion will be extended to a consideration of lignin biosynthesis vis-à-vis O_2 partial pressures and to the possible connection with the explosive radiation of land plants subsequent to the migration. The UV screen concept has already been proposed (Lowry, Lee, and Hebant, 1980), but in a more qualitative sense; I attempt in this chapter to provide a quantitative evaluation.

Characteristics of Land Plants

It is necessary to consider exactly what is meant by the term *land plant*. A strict definition will not be given; instead, a description based on functional considerations will be provided so that the reader will know what groups of plants are encompassed by this term.

The essential difference between aquatic and terrestrial plants is their handling of water relations. Aquatic plants inhabit a water-sufficient environment (those living in hypersaline areas are regarded as specialized derivatives of aquatic plants). Terrestrial plants, in contrast,

live in a water-deficient environment. Their adaptation to this problem of water deficiency is a key feature of hypotheses concerning their migration to the land. This statement implies that the ancestors of the land flora were primarily, or originally, aquatic, and it is accepted that these ancestors were the aquatic green algae.

Fungi in nonaquatic environments also encounter problems of water deficiency and have acquired a number of adaptations to deal with them. As heterotrophs and chemotrophs, however, they use organic carbon as their source of cellular carbon and energy and hence almost certainly appeared on the terrestrial scene after the appearance of a land flora and/or fauna.

In this discussion the term *land plant* will refer to plants permanently adapted to a terrestrial or water-deficient habitat. This is a functional description, designed for this paper. Aquatic plants such as *Myriophyllum*, *Elodea*, *Sagittaria*, and *Potamogeton* are secondarily aquatic having "migrated" back to water and adapted with a secondary reduction of lignified tissue, formation of aerenchyma, and the acquisition of an algalike morphology. Many bryophytes and algae also inhabit damp environments and have acquired some but not all of the adaptations needed for a terrestrial existence; they are restricted to a semiaquatic or semiterrestrial habitat. There are additionally a number of drought-resistant bryophytes and terrestrial (for example, soil) algae.

A plant, to be considered permanently adapted to a terrestrial habitat (Lewis, 1980; Miller, 1970; Stebbins and Hill, 1980; Walker, 1983), must possess the following characteristics.

1. A soil-bound absorbing region (roots or rhizoids).
2. Waterproofing on the aerial parts to keep water in and "toxic" solutions out (Walker, 1983). Waterproofing is provided by the polymer cutin.
3. A water transport system. Water transport is principally the function of the xylem tissue and its associated lignin, which serve also as mechanical support for the aerial part of the plant. In the case of some mosses, however, water transport and conduction is provided by hydroids and leptoids, which are nonlignified tissues.

The *transitional* plant, which can be accepted as semiaquatic, but not permanently adapted to land, would have to meet the first two requirements. A permanently adapted plant (that is, a land plant) would be expected to meet all three.

In this context it should be noted that the term *permanently adapted* does not mean "restricted" but rather "capable of survival of growth."

With these descriptions and caveats in mind, one can include in land plants both vascular plants (such as *Myriophyllum*, *Elodea*, *Sagittaria*, and *Potamogeton*, mentioned above) and bryophytes. Aquatic algae, obviously, do not need to meet these requirements. So-called terrestrial algae circumvent many water-deficit problems with a tolerance of desiccation or a mucilaginous sheath to minimize water loss. Growth of such terrestrial algae is usually very slow.

Other nonessential or secondary adaptations (table 3.1) would

Table 3.1 Adaptation of Algae to a Terrestrial Existence and the Needed Modifications

Green Algae to Semiaquatic Existence

- Development of waterproofing: cutin
- Development of pores for gas exchange: simple pores
- Development of UV shield (in absence of sufficient O₃ shield)
- Development of desiccation-resistant spores: sporopollenin
- Partial substrate-attached absorbing region

Semiaquatic to Totally Terrestrial Existence

- Development of water-conducting tissue: lignin
 - Mechanical support for aerial plants: lignin
 - Soil-bound absorbing region (roots or rhizoids)
 - Mycorrhizal system with roots: fungal association
 - Reproduction independent of swimming gametes or zoospores
 - Reduction of surface-to-volume ratio
 - Improved gas exchange (stomata)
-

include (Jeffrey, 1962; Stebbins and Hill, 1980) the development of a reproductive mechanism independent of swimming gametes or zoospores; a zygotic resting stage on the aerial structure; thick-walled spores and a tendency toward the reduction of the surface-to-volume ratio; and an efficient gas-exchange mechanism. None of these may be considered essential to the migration. They are later developments that contributed to the subsequent adaptive radiation of the land flora. Another important adaption and one that has received little attention is the development of mycotrophism or mycorrhizal associations (Malloch, Pirozynski, and Raven, 1980; Pirozynski and Malloch, 1975). This system, describing terrestrial plants (Pirozynski and Malloch, 1975, 153) as the product of a "continuing symbiosis of a semiaquatic ancestral green alga and an aquatic fungus — an oomycete," is important to the solution of nutrient starvation and is argued to be an important factor in the development of a land flora.

Land Plants and Presumptive Green Algal Ancestors

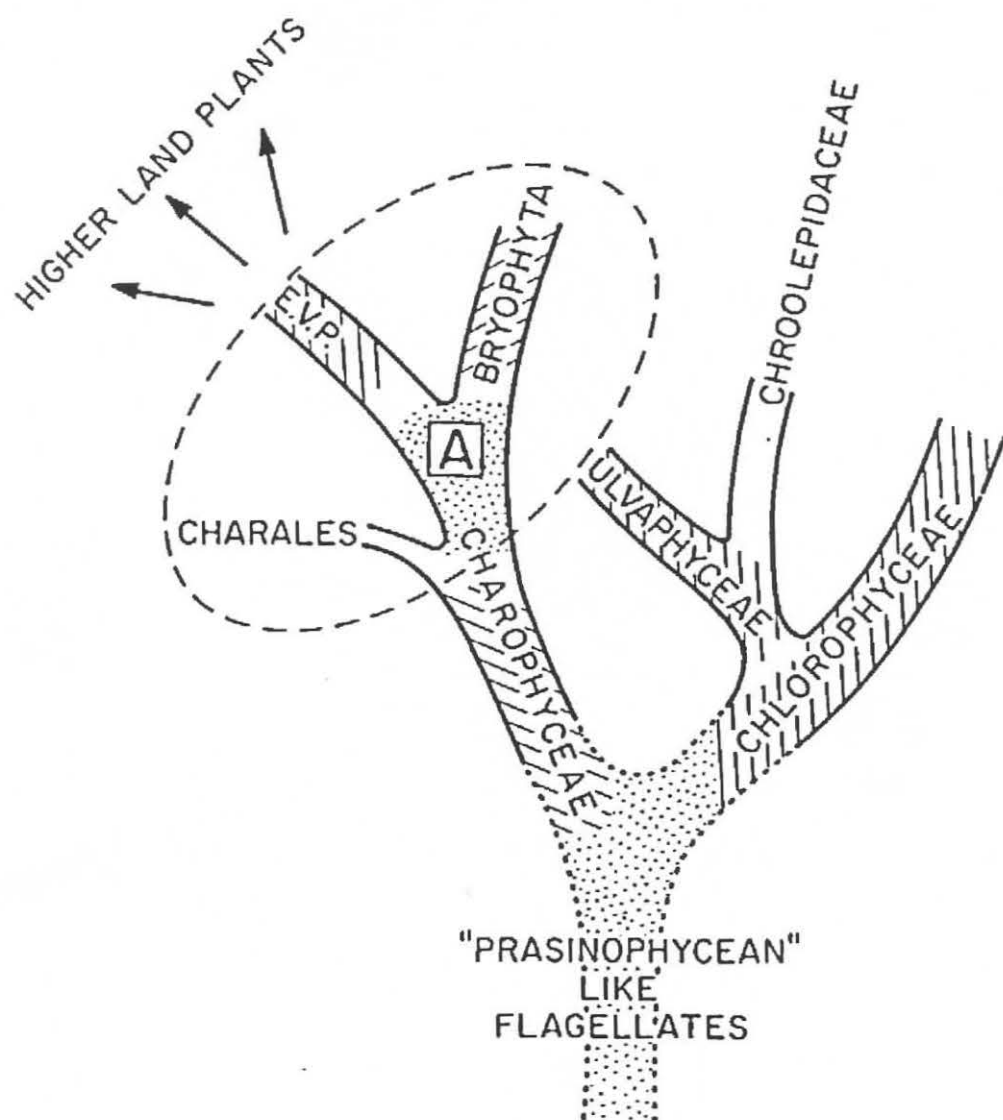
Seventy-five years ago Bower's (1908) classic work, *The Origins of a Land Flora*, was published. In the intervening years two recurrent but essentially unanswered questions have persisted as the sources of considerable speculation: What was the putative algal ancestor of embryophytes and what was the nature of the first land plants?

The postulated algal ancestor was sought usually in the Chaeophorales (for example, *Fritschiella*) — one of the primary reasons for this hypothesis being the heterotrichous habit found in this order, which provided a gross morphological similarity to what many have envisaged as the structure of the primitive plants. At first glance this seems to be a perfectly reasonable assumption. Certainly at the level of gross morphology there are no other candidates that can be considered as "more obvious" ancestors. However, such arguments are prejudiced by the sparse fossil record of identifiable early land plants, views about the phylogenetic position of the Bryophyta, and the morphology of simple extant vascular plants. By any account there is a vast morphological chasm between any putative algal ancestor and any as yet identified primitive land plant, fossil or living.

The last fifteen years have seen a total reevaluation of green algal taxonomy and phylogeny (Melkonian, 1980, 1982; Moestrup, 1978; Pickett-Heaps, 1975; Stewart and Mattox, 1975, 1978). The impetus for this change derives from ultrastructural studies, especially on mitotic and microtubular systems and flagellar root structures, with support from biochemical data. The proposed phylogenetic model is shown in figure 3.1, and a chlorophytan taxonomy is given in table 3.2. When comparisons with higher plants are then made it becomes obvious that

Table 3.2 Taxonomy of the Chlorophyta

Class:	Ulvophyceae
Orders:	Dasycladales
	Codiales
Class:	Chlorophyceae
Genera:	<i>Fritschiella</i>
	<i>Stigeoclonium</i>
Class:	Charophyceae
Orders:	Charales (<i>Chara</i> , <i>Nitella</i>)
	Conjugales (<i>Spirogyra</i>)
	Klebsormidiales
	Coleochaetales (<i>Coleochaete</i>)



E.V.P. = EARLY VASCULAR PLANTS

Fig. 3.1. Phylogenetic scheme of the "green plants." Section inside the ellipse represents the position of the land migration discussed in the text. A represents the evolutionary position of the hypothetical migratory green alga. The various taxa and groups of organisms are delineated by shaded and stippled areas.

the old Chaetophorales must be abandoned as the algal ancestral stock and that more likely candidates are the Klebsormidiales and Col-cochaetales, now in the class Charophyceae (*sensu* Stewart and Mattox, 1975, 1978). Included in the "abandoned" order were such genera as *Fritschella* and *Stigeoclonium*, both of which have a basal and aerial filamentous part to their thalli. The grounds for this rejection are the major differences between these algae and higher plants in flagellar base and root structure (Melkonian, 1982; Stewart and Mattox, 1975, 1978) and mitotic-cytokinetic behavior (Pickett-Heaps, 1975, 1979).

Chaetophoraleans with mitotic spindle, cell division, and flagellar root and base structures similar to those of higher plants have been "transferred" to the Charophyceae, which includes *Chara* and *Nitella* (order Charales). Superficially, this is an unhappy state of affairs. The morphological gap between a primitive presumptive land plant and the green algal stock is now greater. A "best" green algal ancestor now becomes *Coleochaete*, a simple heterotrichous form with a prostrate disc and erect hairs. It would apparently be simpler to formulate hypotheses regarding the ancestors of the vascular land plants if (1) there were a charophycean alga with distinct tissues, an erect thallus, and some form of identifiable (preferably terminal) sporangia or (2) if there were a discernible semiaquatic fossil with a more algalike morphology than *Cooksonia*, a very early fossil vascular land plant. The advanced members of the Charophyceae (Charales) do not meet this requirement and indeed one can argue that they are an evolutionary side branch of the charophyceae, not closely related to the line giving rise to the vascular land plants.

The argument whether unicellular or multicellular green algae were the transitional stock (Stebbins and Hill, 1980) does not affect the discussion pertinent to vascular land plants. It is important in considering nonvascular land plants, and these authors have proposed that the main evolution of the Charophyceae occurred on moist surfaces on land. The extant Charophyceae, which are almost entirely freshwater, would thus be secondarily aquatic. The recovery of *Cooksonia*, from marine sediments in some instances, is not proof of an aquatic habitat.

The Fossil Record and the Time Frame and of the Land Migration

The first land plant, or at least one definitely identifiable as *vascular* and *terrestrial*, is generally accepted (Banks 1975a, 1975b; Taylor, 1982) to be similar to *Cooksonia*. Other primitive plant remains with ligninlike chemistry, but not necessarily vascularized, include *Eohostimella* and *Sciadophyton* (cf. Niklas, 1980); primitive and apparently nonvascularized remains include *Horneophyton*, *Steganotheca*, and *Spongiophyton* (Niklas, 1980; Taylor, 1982). The nonvascular nature of the two former may be doubtful, and proof of a green algal ancestry for *Spongiophyton* is lacking. Indeed, green algal affinities of many nonvascular remains are questionable; consider, for example, the paleobiochemical clustering of such fossils as *Prototaxites*, *Protosalvinia*, and *Nematothallus* with brown and red algae (Niklas, 1980).

Plant remains (for example, *Eohostimella*) with ligninlike biochemistry (vascularized?) have been dated to the lower Silurian (Niklas,

1980; Niklas and Pratt, 1980), 475 million years ago (cf. also Schopf, 1970). *Cooksonia*, the most commonly accepted first vascular land plant, dates back to the upper Silurian, 405 million years ago. The reader is referred to Edwards, Bassett, and Rogerson (1979) for a discussion of the earliest *Cooksonia*.

Nonvascularized remains have been recorded from the lower (Pratt, Phillips, and Dennison, 1978; Niklas and Smocovitis, 1983; Strother and Traverse, 1979) and upper Silurian. Perhaps the most notable of these is *Parka*, which can be regarded as showing morphological (Niklas, 1976b) and paleobiochemical similarities (Niklas, 1976a) with hypothetical ancestral green algae. Fossil green algae have been identified in the late Cambrian-Ordovician (Wray, 1977), 550–500 million years ago. These forms are advanced Dasycladales and Codiales (Herak, Kochansky-Devide, and Gusic, 1977), two orders that are not in the general evolutionary line to the higher plants. The ancestral stock (the charophytes) has representatives dating to the upper Silurian (Grambast, 1974). Assumed green algae are generally considered to be much older, but it is difficult to establish their true affinities vis-à-vis the Charophyceae, Chlorophyceae, or Ulvophyceae. The net result of this fossil record is to suggest (Taylor, 1982, 171) that “land inhabiting plants were well established by the beginning of the Silurian, and possibly much earlier in the Ordovician.” It is important to remember that the dates of the fossil record represent only the first *identification*, not the first actual occurrence, which may well have been earlier. In the remainder of this paper I will attempt to show that these data, and those for vascularization (dated to the late Silurian, 415 million years ago), both established from the fossil record, agree with the dates developed from biochemical/environmental arguments, which are based upon the premises, outlined in the introduction concerning the role of the ultraviolet screen and an oxygenated atmosphere needed to produce this screen and to support certain biochemistries essential for the establishment of a land flora.

Biochemical Requirements and the Environment

The arguments outlined above suggest that the migration to the land and the adaptation to the terrestrial habitat required two major *biochemical* innovations not required by (or found in) the presumptive green algal aquatic ancestor: (1) development of cutin and (2) elaboration of phenylpropanoid metabolism. The first meets the requirement for a waterproofing agent. Phenylpropanoid metabolism could have provided a solution to the potential need for an ultraviolet screen.

Modeling of atmospheric changes over geological time (see below) suggests that the ozone screen may have been insufficiently developed at the time of the transition (perhaps 600 million years ago) to provide an "external" UV screen for the emerging land flora. If this were the case, then one can argue that the transition would have been dependent in part upon, or facilitated by, the development of an "internal" (plant-originated) UV screen. This assumption has been incorporated into the material of this paper. Although lignin is not essential for the transition, its subsequent appearance, which provided for the morphological elaboration of erect structures and production of water-conducting xylem tissue, presumably soon after the transition, was important in the elaboration of the land flora. The reader is referred to two recent publications (Niklas, 1976c, 1982) for a discussion of possible additional roles for these and other secondary metabolites. The question remains, were the environmental changes that may have been occurring at the time of the transition instrumental in facilitating these innovations?

Cutin: Waterproofing and Protection

Waterproofing and protection against external toxic solutes is the *raison d'être* for cutin. In aquatic systems waterproofing is not a real problem, and toxic substances can be expected to be diluted. Protective coatings can also be provided by heavy mucilaginous layers, thick cellulose walls, or sporopollenin. However, cutin appears to be the best waterproofing agent. Besides its excellent water-repellent properties, it lacks the rigidity of sporopollenin but, unlike mucilage, it is rigid enough to allow pores (stomata) to open and close for critical gas exchange.

Cutin is composed of a phenylpropanoid, C_{16} (or C_{18}) polyoxy fatty acid polymer (Kolattukudy, 1980). The structure of cutin reveals a polymer whose monomeric unit is coumaric acid complexed to a number of mono- and dihydroxy C_{16} or C_{18} fatty acids. The hydroxylation-epoxidation of the fatty acid requires O_2 . The synthesis of the hydroxy-palmitate (C_{16}) moiety involves two hydroxylations ($NADPH/O_2$) and that of the trihydroxystearate (C_{18}) involves three similar oxygenation steps. Cutin is a highly oxygenated substance, as are the phenylpropanoids, and is essential to land-plant development.

The migratory alga, or the very first semiaquatics, would have had to develop the capacity to synthesize cutin very early. Besides acting as a waterproofing agent, cutin can also be regarded as playing a protective role (Walker, 1983) — keeping toxic or harmful solutions out. No algae have been identified as synthesizing cutin (they really have no need for it), whereas the semiaquatic or terrestrial mosses and liverworts (Kolatt-

tukudy, 1980, 1981) in the Bryophyta do produce the substance. The retention of cutin in aquatic angiosperms is obviously a residual trait retained during the recolonization of the aquatic environment.

There is currently no information about the partial pressure of O_2 needed for cutin biosynthesis, either for the total compound or the individual coumaric acid and hydroxy fatty acids. What information is available (Chapman and Schopf, 1983) suggests that there is generally a critical level of O_2 partial pressure for an individual enzymatic reaction (called systemic biochemistry) and another critical level for the maintenance of organismic or physiological functions, or a complete biosynthetic sequence of serial enzymatic steps. The O_2 concentration necessary for half-maximal activity of individual aerobic (O_2 -requiring) enzymes that have been examined (see citations in Chapman and Schopf, 1983) is 0.002 PAL O_2 (the PAL, or present atmospheric level, of O_2 is 0.2 atmospheres).

The half-maximal O_2 partial pressure necessary to produce a full cellular complement (that is, concentrations normally encountered) of oxygenated compounds requiring oxygen in biosynthesis is 4×10^{-4} atmospheres (= 0.002 PAL O_2). Physiological functions (growth, respiration, mitosis) show half-maximal activity (or inhibition, in the case of mitosis) at about 0.02 PAL O_2 — an order of magnitude higher. The oxygen level at which amphiaerobic organisms (Chapman and Schopf, 1983) switch from an anaerobic to an aerobic metabolism (Pasteur point) is approximately 0.01 PAL O_2 . These values suggest that although individual biosynthetic steps in coumaric acid synthesis and fatty acid hydroxylation may have been operative at lower O_2 partial pressures, aggregate pathways involving a number of O_2 -requiring steps might not have occurred until the level was in the range of 0.02 PAL O_2 . With the exception of certain carotenoids in the chloroplasts, biosynthetic sequences involving numerous O_2 -requiring steps are not common in green algae. It is in the land plants that one usually finds the elaboration of polyoxygen metabolites, in which the O_2 -requiring steps frequently occur as "terminal add-ons" to preexisting pathways.

Let us assume that an O_2 level of 0.02 PAL is needed for cutin biosynthesis. At what time in the past was this level reached? Estimates of oxygen levels are based on geological and paleobiological data and on atmospheric modeling. These estimates are usually given only as limits (maximum or minimum, earliest or latest) within which one can work. Walker et al. (1983) have argued that it is unlikely that an oxygen level of 0.1 PAL was reached prior to 1.7 Ga ago and that in fact a stable aerobic environment was established apparently no earlier than 1.7 Ga

ago. These are *not* the dates of the attainment of this O₂ level but only the earliest possible date. Rhoads and Morse (1971) have examined the metazoan fossil record in the light of modern metazoan distribution in low-O₂ environments. They have suggested that an O₂ level of 0.01 PAL was reached about 0.7–0.8 Ga ago and a minimum of 0.1 PAL was reached at the lower Cambrian boundary of 570 million years ago. These sets of dates provide a window, admittedly broad, within which the migration could have occurred. The dates provided by Rhoads and Morse (1971) coincide quite well with the fossil record which indicates the presence of land flora approximately 500–450 million years ago and a presumably earlier date for the transition to the land.

Phenylpropanoids and UV Screening

A land migration could not have occurred until either sufficient ozone existed to screen out the UV irradiation or the antecedents to the migratory flora had developed their own internal UV screen. Although O₂-O₃ calculations (see below) would suggest that an internally produced (in vivo) UV screen was not necessary, such a screen may well have facilitated the migration if the O₃ levels at the time were marginal or had just attained the appropriate level. Several compounds typical of C₆-C₃ metabolism have good UV-absorbing properties, and it has previously been suggested (Lowry, Lee, and Hebant, 1980) that phenylpropanoid metabolism did in fact represent an early protective adaptation. The simplest UV-absorbing compounds are coumaric acid and its immediate derivatives. We can assume coumaric acid, as an intermediate to the ubiquinones, was formed in all organisms. Formation of its hydroxy derivatives in the land-plant antecedents has not been demonstrated. These derivatives have some potential as UV screens, but there are some unanswered questions.

Examples of compounds that screen UV light include the following.

	Absorption Spectrum λ max in nm (methanol)
Coniferyl alcohol	265
Dihydroxy dimethoxy stilbene	306–333
Coumaric acid	224–299
Ferulic acid	235–320
Sinapic acid	240–320

These substances are derivatives of phenylalanine and their formations are governed by a key enzyme, phenylalanine ammonia lyase. This enzyme has been detected in the chlorophycean *Dunaliella* and its

presence may be assumed by the synthesis of ubiquinone in algae. We do not know to what extent the simple coumaric acid derivatives are stored free in the cell, as opposed to serving as intermediary metabolites for lignins, and dilignols of bryophytes. It may be that they themselves are insignificant in UV protection and that the important compounds would have been the flavonoids. The presence of flavonoids (Markham and Porter, 1969; Swain, 1980), elaborated from C₆-C₃ phenylpropanoids, has been observed in *Nitella* and *Chara* (Charales). Flavonoids have not been reported in any other green algae, so their presence in *Nitella* and *Chara* suggests that flavonoid production may be a feature of the advanced members of the Charophyceae, the group that presumably gave rise to higher plants. Considerably more comparative biochemical investigation is needed to offset the caveat that this distribution may be simply a reflection of the extent of the search. There is good documentation that bryophytes have the capacity to elaborate simple phenylpropanoids. This is shown by the capacity of bryophytes to synthesize lunularic acid (Gorham, 1977a, 1977b; Pryce, 1972) and a wide selection of flavonoids (Markham and Porter, 1978). It would be interesting to look for lunularic acid in the Charophyceae line. The algae examined by Gorham (1977a; cf. Pryce, 1972) are members of the Chlorophyceae and Ulvophyceae, not on the line to higher plants. Figure 3.2 illustrates the basic pathways of phenylpropanoid metabolism, and figure 3.3 shows the structures of lunularic acid and two representative flavonoids found in bryophytes and *Nitella*. Many flavonoids, including those in *Nitella*, have UV-absorbing properties that could well enable them to serve as effective UV screens, provided there was an appropriate cellular location and content, a feature that is not known for either *Nitella* or the bryophytes.

UV-Absorption Spectra Properties of *Nitella* Flavonoids

λ max in nm (methanol)

Lucenin	256–270
Orientin	255–267
Vicenin	273–333
Vitexin	270–336

These data from comparative biochemistry, admittedly sparse, do suggest that the putative ancestors of the migratory flora were capable of synthesizing their own UV screen, in the form of flavonoids, and that these compounds were probably present in the earliest land plants.

Two questions must now be resolved concerning the timing of the

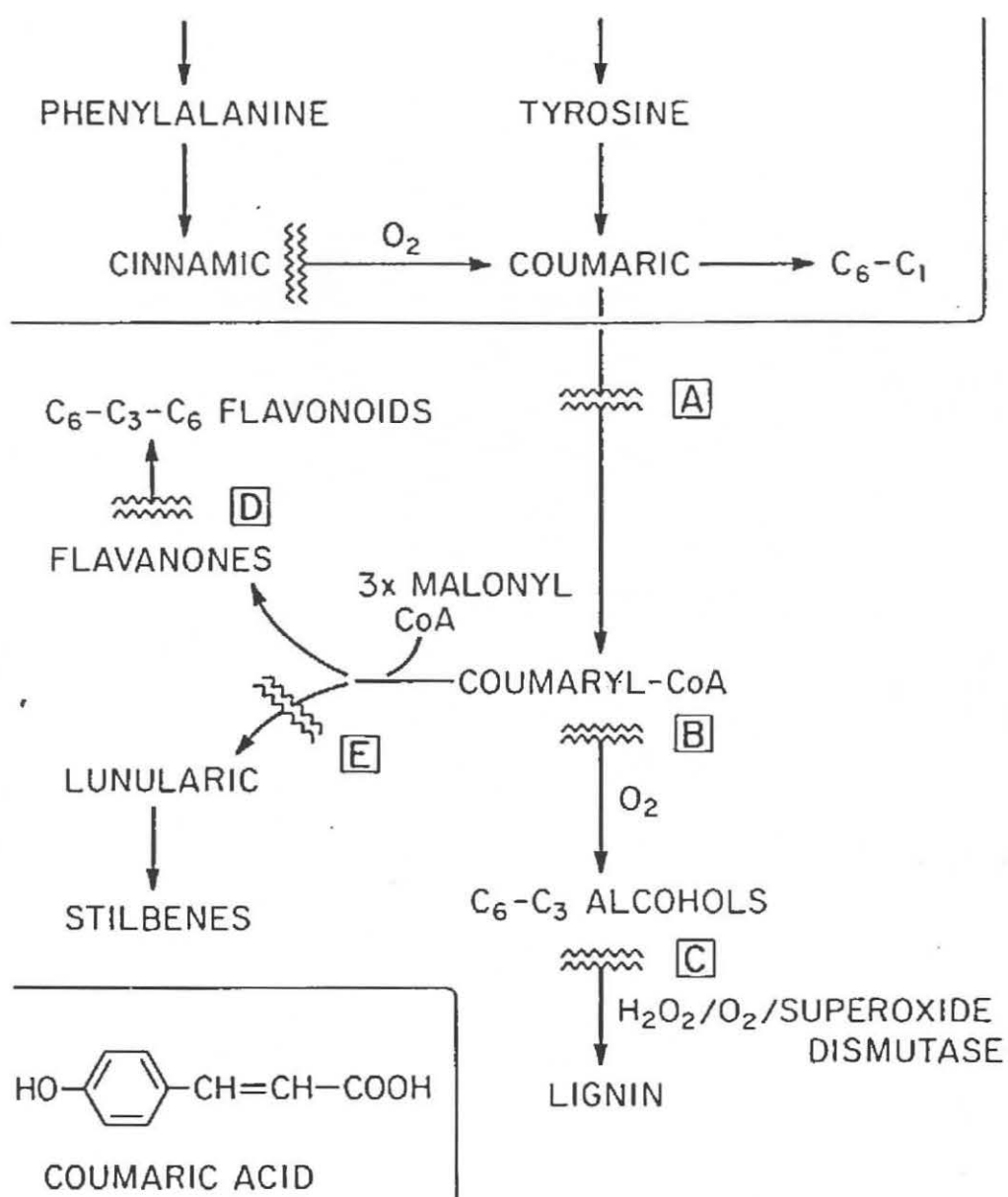
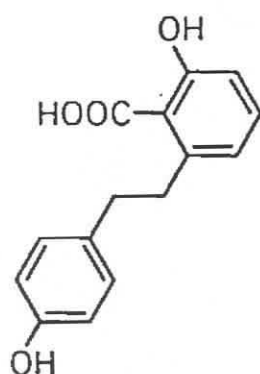


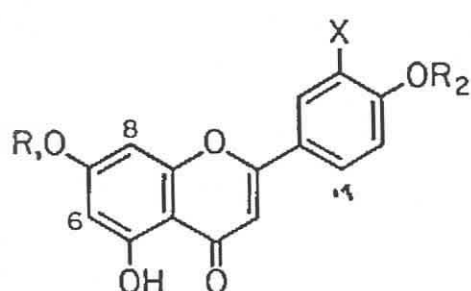
Fig. 3.2. Phenylpropanoid metabolism. Area inside the line at the top represents metabolism common to all groups.

- A. Coumaryl ligase. Appearance of this enzyme enabled the biosynthesis of flavanones and stilbenes and the establishment of an initial internal ultraviolet screen.
- B. Monophenol oxygenase. This enzyme is necessary for the elaboration of the C_6-C_3 alcohols, an O_2 -requiring step. The alcohols are precursors of lignin and the dimers of bryophytes.
- C. Elaboration to the true lignins of higher plants representing a complex of steps requiring superoxide, H_2O_2 , and peroxidase.
- D. Flavanone elaboration to the family of flavones.
- E. Extension of A, which allowed synthesis of stilbenes in bryophytes. This is a presumptive biosynthetic pathway.



LUNULARIC ACID

In BRYOPHYTA: HEPATICOPSIDA



FLAVANONE SKELETON

In: CHAROPHYCEAE
BRYOPHYTA
(BRYOPSIDA)
(HEPATICOPSIDA)

Apigenin: $R_1 = R_2 = X = H$

Vicenin: C-6, C-8 Diglycoside

Vitexin: C-8 Monoglycoside

Luteolin: $R_1 = R_2 = H$; $X = OH$

Lucenin: C-6, C-8 Diglycoside

Orientin: C-8 Monoglycoside

Fig. 3.3. An example of the stilbenes (lunularic acid) and skeletal structure of the flavanones, which are synthesized by the Bryophyta and Charophyceae. These compounds may have represented early internal, or cellular, screens to UV radiation. Synthesis of these compounds is indicated by step A in figure 3.2.

invasion of the land. First, was a sufficient ozone screen present at that time, and if not, was an internal UV screen present in the earliest land plants? Second, what is the time frame of the appearance of these two features? Calculations of early ozone levels depend upon theoretical calculations of modern ozone production and upon the estimation of early oxygen levels, which are based upon calculations and estimates from the geological record. Walker et al., (1983) have pointed out the disparity in the various estimates but have suggested that the calcula-

tions of Kasting and Donahue (1980), who reevaluated the earlier calculations of other workers, are the most reliable. Kasting and Donahue found that an effective UV screen does not occur until an O_2 level of 0.1 PAL is reached. In the absence of a cellular UV screen (for example, flavonoids or other phenylpropanoids), a land migration is unlikely to have occurred prior to a time at which this level was reached. Walker et al. (1983) have indicated that it is unlikely that a level of 0.1 PAL O_2 was reached before 1.7 Ga ago. However, as mentioned earlier in the discussion of cutin, this represents an "earliest possible" date. In contrast, Rhoads and Morse (1971) have suggested from paleobiological considerations that a minimum of 0.1 PAL was reached at the lower Cambrian boundary of 570 million years ago.

Turning again to the option of an internal UV screen, we must still consider the time frame. There is an interesting catch to the biosynthesis of phenylpropanoids that bears on this evolutionary development. The biosynthesis of the polyhydroxy C_6-C_3 phenylpropanoids requires oxygen (fig. 3.2). Even though ammonia lyase activity may well have been operative in early green algae (*viz.*, phenylalanine ammonia lyase activity in *Dunaliella*; Loeffelhardt, Ludwig, and Kindl, 1973), elaboration of the aromatic amino acid phenylalanine to its polyhydroxy derivatives presumably would have been influenced by O_2 partial pressures. The same arguments for a minimal O_2 partial pressure that were used for cutin biosynthesis (see above) can be applied here. In other words, although individual steps in the biosynthesis of the polyhydroxy phenylpropanoids may operate at low O_2 levels (0.002 PAL), aggregate pathways to a polyhydroxy compound (for example, flavonoid) may require higher levels of about 0.02 PAL O_2 .

An internal screen (for example, the presence of phenylpropanoids) is not universal among simple plants. It is frequently absent in fungi, and many green algae (those not on the line to the higher plants; that is, Chlorophyceae, Ulvophyceae) may lack such a screen. Does this favor the option that an efficient O_3 screen was already present prior to the migration? Or, alternatively, does it suggest that there may have been later, continued migrations after the attainment of an O_3 screen? At this time it is probably impossible to distinguish between these two alternatives. For example, we know very little about mycorrhizal fungi and their capacity to synthesize potential internal chemical UV screens or their time of migration. It must also be remembered that the land migration may not have been a "one-event" occurrence and that a number of migratory "events" may have occurred while the O_3 shield was increasing; some organisms may have been developing an internal shield and some not.

Lignin: Water Transport and Support

Lignin, which is a polymer of C₆-C₃ phenylpropanoid compounds, is the least essential of the three requisites under consideration for land-plant colonization. Lignin is not required for the migration and the development of land plants with aerial parts. The presence of lignin surely contributed to the full evolution of the land flora by providing structural rigidity and the lignified vascular tissue. There is no evidence to suggest that the transitional plants could synthesize lignin, although they almost certainly could synthesize its precursors. Advanced green algae of the Charophyceae had acquired the capacity to synthesize C₆-C₃ phenylpropanoid precursors, as shown by the synthesis of flavonoids (Lowry, Lee, and Hebant, 1980; Swain, 1980) in *Nitella* and *Chara*. Green algae of the Chlorophyceae and Ulvophyceae, which are not on the line to higher plants, apparently have not acquired the capacity to elaborate C₆-C₃ metabolism beyond coumaric acid (see fig. 3.2), as shown by the apparent failure to identify later biosynthetic compounds in these groups. Bryophytes can synthesize lunularic acid (Gorham, 1977a, 1977b; Pryce, 1972) and have, additionally, acquired the ability to modify the C₆-C₃ moiety and dimerize it (Miksche and Yasuda, 1978). These dilignols, however, are *not* true lignins.

Lignin formation is the one process of those being considered that is most highly dependent on oxygen. In addition to the usual oxygenation steps for phenylpropanoid elaboration, the subsequent oxyradical formation (Gross, 1980; Gross, Janse, and Elstner, 1977; Halliwell, 1978) involves the transformation of oxygen to superoxide. The disproportionation of superoxide gives hydrogen peroxide, which is utilized (via peroxidase) to yield the key oxyphenylpropanoid radical. The subsequent polymerization appears to be nonenzymatic. Presumably lignin formation could not proceed and could not have developed in the presence of low O₂ partial pressures. It is reasonable to assume, from the earlier discussions on cutin and phenylpropanoids, that partial pressures of O₂ would need to be in excess of 0.004 atmospheres, or 0.02 PAL O₂, for lignin formation. One of the more interesting features of lignin formation is the involvement of superoxide dismutase. Although the nature of the superoxide dismutase (SOD) involved in the reaction is unknown, it is tempting to speculate that it is the cytosol Cu/Zn form rather than the mitochondrial Mn/SOD or Fe/SOD. The Cu/Zn SOD is distributed between higher land plants, charophycean green algae (*Nitella*, *Spirogyra*), fungi, and animals — a distribution that admittedly poses some interesting phylogenetic problems. It has not been identified in prokaryotes, other algae, or green algae of the chlorophycean or

ulvaphycean lineage (see Chapman and Schopf, 1983, and citations therein). It seems reasonable to speculate, certainly from a basis of comparative biochemistry, that the appearance of Cu/Zn superoxide dismutase appeared as a response to rising O_2 levels and a need to protect against internal oxygen toxicity from direct exposure to atmospheric O_2 in place of dissolved O_2 . In the case of land plants the protective mechanism — and its byproduct, hydrogen peroxide — has been incorporated into the synthesis of lignin. I would suggest that the oxygen requirements for lignin biosynthesis (in terms of partial pressure) must be at least as high, if not higher, than those for cutin formation and general phenylpropanoid metabolism and elaboration. If this is the case then one can reason that the early transitional or semiaquatic plants were perhaps incapable of lignin biosynthesis. The increasing oxygen partial pressures (which continued to increase after the migration) subsequently allowed for lignin biosynthesis; hence the vascularized land plants could have evolved only after the initial migration. This scenario is, of course, dependent upon the assumption of a late date for the appearance of O_2 levels in the range 0.01–0.1 PAL.

Evolutionary Position of the Bryophytes: Nature of the Migratory Land Plant

Early views often held that bryophytes, because their simplified morphology and partial adaptation to a terrestrial habitat, represented an early *ancestral* land-plant stock. The current prevailing opinion (Remy, 1982; Schuster, 1979; Smith, 1978), as illustrated in figures 3.1 and 3.2, holds that bryophytes and tracheophytes diverged from a common stock. This hypothetical ancestral group may thus represent the transitional flora. The nature of these organisms, especially with regard to their biochemistry (for example, the formation of lignins and cutins), pertinent to water supply and retention is critical to arguments developed in this chapter. It has been pointed out (Miller, 1970; Raven, 1977) that a *successful inhabitation* of the land depended upon an internal water-conducting system. The fossil and paleobiochemical record of the vascular land plants, bryophytes (offshoots of the transitional or first semiaquatic plants, which were cutinized but not vascularized), and the ancestral stock to both of these (the first semiaquatics?; Raven, 1977) is critical to time correlations.

Other Hypotheses

It is not my intention to suggest that an oxygen link was the *prime* control of the timing of the invasion of the land, only that it may well

have been a component, provided that certain assumptions are made. Oxygen, or rather the lack of it, may have acted as a restraint on a potential land migration and development of vascularized plants. In recent years a number of other hypotheses have been discussed. The UV-phenylpropanoid connection has already been considered (Lowry, Lee, and Hebant, 1980) as an essential feature of a land migration. As this paper reemphasizes, UV protection, which is linked to O₂ levels through O₃ levels, may well have been a critical factor. Raven (1977) has recently considered the problem from the point of view of maintaining a homoiohydric condition (that is, the capacity to remain hydrated under circumstances of limited water supply). There is no question that the development of such a capacity was essential for the appearance of land plants. The evolution of lignin and cutin is an integral feature for the acquisition of this homoiohydric state. Raven has emphasized this and points out that some green algae show preadaptation along these lines. In a more provocative article, Lewis (1980) has suggested a key role for borate in the hydroxylase and oxidase steps in lignin biosynthesis. The origin of this role depended upon the selection for sucrose (a weak complexer with borate), rather than acyclic polyols, which bind and sequester borate, in the Chlorophyta. Without dismissing Lewis's hypotheses, one must remember we know very little about the function of borate in plant cells. If green algae (Charophyceae) were preadapted for phenylpropanoids and had selected for sucrose biosynthesis, one can ask why there has not been a greater elaboration of C₆-C₃ metabolism in this group. Borate is certainly abundant in aquatic systems.

Semiaquatics and presumably the ancestral migratory flora in moist environments are not subject to the potential problems of nutrient deficiency that plague strictly terrestrial plants. The role of mycorrhizal symbioses in the subsequent adaptation of semiaquatics and the development of a truly terrestrial flora is probably very important and reflects the need of a terrestrial plant to overcome the problems of nutrient shortage.

In the final analysis, however, the land migration and subsequent terrestrial development surely reflect an aggregate of a number of factors, some of which may have been environmental.

Proof and Conjecture

In this chapter oxygen, acting as a restraint mechanism in a number of ways, has been proposed as a contributing factor in the evolution of a land flora. The suggestions, however, depend upon a knowledge of the rate of increase of O₂ in the atmosphere and the time frame. At the biochemical level one is interested in two O₂ levels, approximately 0.002

PAL and 0.02 PAL; for the attainment of an external ultraviolet screen the level is 0.1 PAL. Calculations, on one hand, have indicated that the earliest *possible* date for 0.1 PAL O_2 was 1.7 Ga ago (Walker et al., 1983; cf. Canuto et al., 1982), whereas paleobiological data (Rhoads and Morse, 1971), on the other hand, have been used to estimate a much later date, 700–800 million years ago for 0.01 PAL and 570 million years ago for 0.1 PAL. If the former estimates are the more accurate, then hypotheses involving UV screens or the role of O_2 in biosyntheses such as outlined here are probably null and void. There is no fossil evidence for a land flora or land transition at that early date. If the latter dates are the more reasonable, then the hypotheses outlined in this paper should be considered; these theories are illustrated in figure

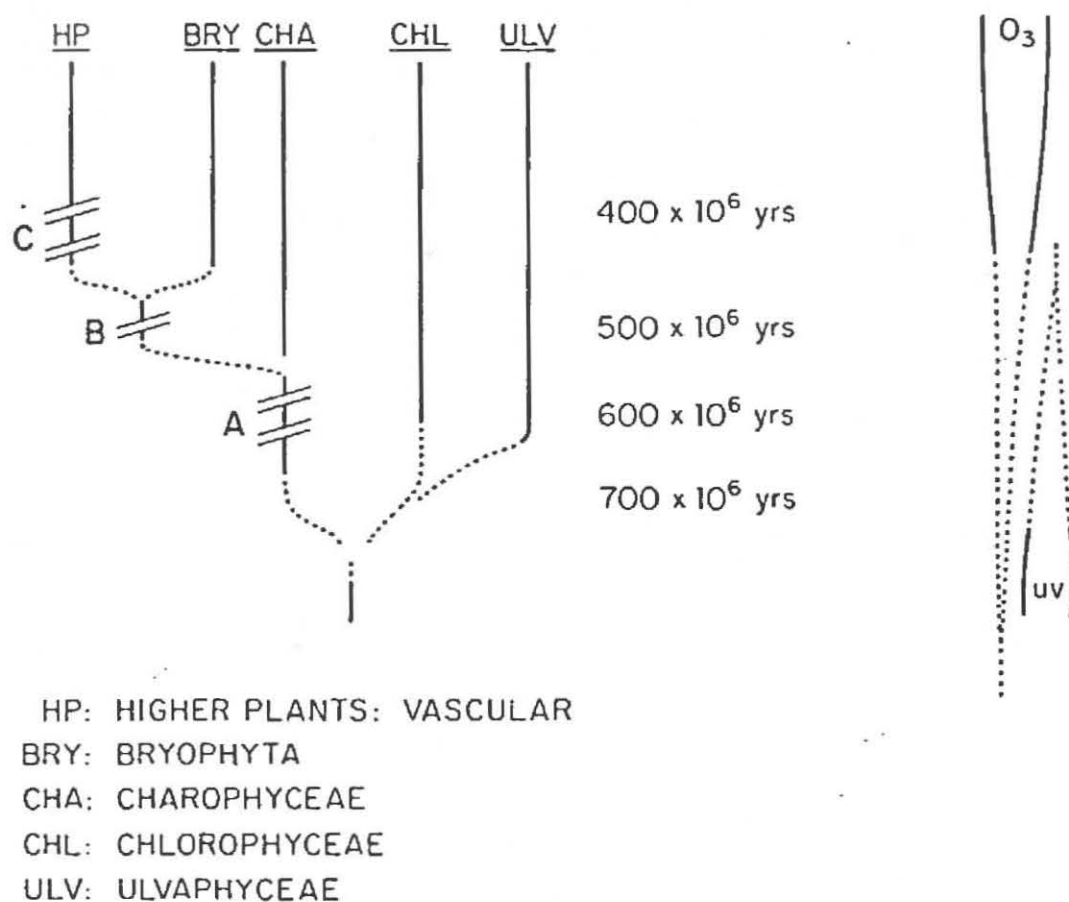


Fig. 3.4. Schematic diagram relating evolution to the biochemical and environmental changes, as suggested by the "late scenario" for O_2/O_3 appearance. The change in width of the O_3 and ultraviolet radiation bars is relative and is designed to represent a possible time frame of appearance and disappearance only.

A. Appearance of Cu/Zn superoxide dismutase and phenylpropanoid elaborations.
 B. Appearance of cutin.
 C. Appearance of lignin and suberin.

3.4. In this regard I quote Kasting and Donahue (1980, 3255) in their introduction: "A question of particular interest is whether or not the development of the ozone layer was responsible for the emergence of life on land during the late Silurian period, about 420 m.y. (million years) ago." These authors conclude that "the uncertainty inherent in both calculations [of earliest and late limits], however, leaves open the possibility that the ultraviolet shield developed after the Cambrian was directly linked with the spread of life onto land" (3261). It is also important to remember that the fossil record pertaining to the land transition, which is sparse and in many instances difficult to interpret, is not a promise that a particular event occurred at a particular time. More important, though, is the question, can these hypotheses be tested? A number of experimental observations could provide data for their validity. These should include investigations on the effect of O_2 partial pressure between 0.001 PAL and 0.1 PAL on the activity of key enzymes in lignin biosynthesis (cinnamic hydroxylase, tyrosinase, peroxidase), superoxide formation, and aggregate lignin biosynthesis; in cutin biosynthesis (fatty acid dehydrogenase, hydroxylase, and epoxidase) and total cutin biosynthesis. Geochemically based and theoretical calculations of O_2 partial pressures and the rate of increase between one Ga and 300 million years ago are needed. To avoid the pitfall of a circular argument, these must be independent of any biological input. Gaps in our knowledge of comparative biochemistry need closing. Specifically, we need to look to the bryophytes and Charophyceae with respect to Cu/Zn superoxide dismutase, peroxidase, phenylalanine ammonia lyase, cinnamic hydroxylase, and general phenylpropanoid metabolism. The solution to such fundamental evolutionary questions as the origin of the land flora will come only from a multidisciplinary approach with input from a variety of sources.

Summary

The origin of a land flora is discussed from a number of viewpoints. A distinction is made between the migration to land (resulting in semi-aquatic plants) and the development of a terrestrial flora (that with lignin for vascularization and cutin for waterproofing). The role of oxygen plays a central part in the discussion, which includes a consideration of the development of an ozone atmosphere and UV shield and the possible need for early land plants to protect against UV irradiation. Following Lowry, Lee, and Hebert (1980), I propose that dependence on O_2 for the synthesis of phenylpropanoid (C_6-C_3) compounds played a part in the timing of the invasion of the land. I suggest that the

O₂-dependent biosynthesis of lignin (for mechanical support and water transport) and cutin (for waterproofing and shielding from toxic solutes) may be related to O₂ partial pressures. Time calculations for the origin of a land flora and the preceding migration are derived from biochemical-geochemical data. The timing of these events compares well with the time frame suggested by the fossil record. I propose that the changing environment, and particularly changes in O₂ partial pressures, played a key part in the migration of the Charophyceae to land and the subsequent development of a land flora.

Acknowledgments

I am indebted to Professors J. W. Schopf and D. B. Walker for their comments on the manuscript, and to the reviewers, Professors A. Knoll, K. Niklas, and B. Tiffney, for their in-depth reading and criticisms, especially with regard to paleobiological matters. To all these people I express my thanks for their unbiased and critical appraisals.

References

- Banks, H. P. 1975a. Early vascular land plants: Proof and conjecture. *Bioscience* 25:730-37.
- . 1975b. The oldest vascular plants: A note of caution. *Rev. Palaeobot. Palynol.* 20:13-25.
- Bower, F. O. 1908. *The origins of a land flora*. London: Macmillan.
- Chapman, D. J., and J. W. Schopf. 1983. Biological and biochemical effects of the development of an aerobic atmosphere. In *Origin and evolution of the earth's earliest biosphere*, ed. J. W. Schopf, 302-20. Princeton: Princeton Univ. Press.
- Edwards, D., M. G. Bassett, and E. C. W. Rogerson. 1979. The earliest vascular land plants: Continuing the search for proof. *Lethaia* 12:313-24.
- Gorham, J. 1977a. Lunularic acid and related compounds in liverworts, algae and *Hydrangea*. *Phytochem.* 16:249-53.
- . 1977b. Metabolism of lunularic acid in liverworts. *Phytochem.* 16:915-18.
- Grambast, L. J. 1974. Phylogeny of the Charophyta. *Taxon* 23:463-81.
- Gray, J., and A. J. Boucot. 1977. Early vascular land plants: Proof and conjecture. *Lethaia* 10:145-74.
- Gross, G. G. 1980. The biochemistry of lignification. *Adv. Bot. Res.* 8:25-63.
- Gross, G. G., C. Janse, and E. F. Elstner. 1977. Involvement of malate, monophenols, and the superoxide radical in hydrogen peroxide formation by isolated cell walls from horseradish (*Armoracia lapathifolia* Gilib). *Planta* 136:271-76.
- Halliwell, B. 1978. Lignin biosynthesis: The generations of hydrogen peroxide

- and superoxide by horseradish peroxidase and its stimulation by manganese (II) and phenols. *Planta* 140:81-88.
- Hebant, C. 1979. Conducting tissues in bryophyte systematics. In *Bryophyte systematics*, ed. G. C. S. Clarke and J. G. Duckett, 365-83. London and New York: Academic Press.
- Herak, M., V. Kochansky-Devide, and I. Gusic. 1977. The development of the Dasyclad algae through the ages. In *Fossil algae*, ed. E. Flugel, 143-53. Berlin: Springer-Verlag.
- Jeffrey, C. 1962. The origin and differentiation of the archegoniate land-plants. *Bot. Notiser* 115:446-54.
- Kasting, J. F., and T. M. Donahue. 1980. The evolution of atmospheric ozone. *J. Geophys. Res.* 85:3255-63.
- Kolattukudy, P. E. 1980. Biopolyester membranes of plants: Cutin and suberin. *Science* 208:990-1000.
- . 1981. Structure, biosynthesis and biodegradation of cutin and suberin. *Ann. Rev. Plant Physiol.* 32:539-67.
- Lewis, D. H. 1980. Boron, lignification and the origin of vascular plants — A unified hypothesis. *New Phytol.* 80:209-29.
- Loeffelhardt, W., B. Ludwig, and H. Kindl. 1973. Thylakoid-gebundene L-Phenylalanin-Ammoniak-Lyase. *Hoppe-Seyler's Z. Physiol. Chem.* 354: 1006-12.
- Lowry, B., D. Lee, and C. Hebant. 1980. The origin of land plants: A new look at an old problem. *Taxon* 29:182-97.
- Malloch, D. W., K. A. Pirozynski, and P. H. Raven. 1980. Ecological and evolutionary significance of mycorrhizal symbioses in vascular plants (A review). *Proc. Natl. Acad. Sci. USA* 77:2113-18.
- Markham, K. R., and L. J. Porter. 1969. Flavonoids in the green algae (Chlorophyta). *Phytochem.* 8:1777-81.
- . 1978. Chemical constituents of the bryophytes. *Prog. Phytochem.* 5:181-272.
- Melkonian, M. 1980. Ultrastructural aspects of basal body associated fibrous structures in green algae: A critical review. *BioSystems* 12:85-104.
- . 1982. Structural and evolutionary aspects of the flagellar apparatus in green algae and land plants. *Taxon* 31:255-65.
- Miksche, G. E., and S. Yasuda. 1978. Lignin of giant mosses and some related species. *Phytochem.* 17:503-04.
- Miller, H. A. 1970. The phylogeny and distribution of the Musci. In *Bryophyte systematics*, ed. G. C. S. Clarke and J. G. Duckett, 11-40. London and New York: Academic Press.
- Moestrup, O. 1978. On the phylogenetic validity of the flagellar apparatus in green algae and other chlorophyll a and b containing plants. *BioSystems* 10:117-44.
- Niklas, K. 1976a. The chemotaxonomy of *Parka decipiens* from the Lower Old Red Sandstone, Scotland (U.K.). *Rev. Palaeobot. Palynol.* 21:205-17.
- . 1976b. Morphological and ontogenetic reconstruction of *Parka decipiens* Fleming and *Pachytheca* Hooker from the Lower Old Red Sandstone,

- Scotland. *Trans. — R. Soc. Edin.* 69:483–99.
- . 1976c. The role of morphological biochemical reciprocity in early land plant evolution. *Ann. Bot.* 40:1239–54.
- . 1980. Paleobiochemical techniques and their applications to paleobotany. *Prog. Phytochem.* 7:143–81.
- . 1982. Chemical diversification and evolution of plants as inferred from paleobiochemical studies. In *Biochemical aspects of evolutionary biology*, ed. M. H. Nitecki, 29–91. Chicago: Univ. of Chicago Press.
- Niklas, K., and L. M. Pratt. 1980. Evidence for lignin-like constituents in early Silurian (Llandoveryan) plant fossils. *Science* 209:396–97.
- Niklas, K., and V. Smocovitis. 1983. Evidence for a conducting strand in Early Silurian (Llandoveryan) plants: Implications for the evolution of land plants. *Palaeobiology* 12:126–37.
- Niklas, K., B. H. Tiffney, and A. H. Knoll. 1980. Apparent changes in the diversity of fossil plants: A preliminary assessment. *Evol. Biol.* 12:1–89.
- Pickett-Heaps, J. D. 1975. *Green algae: Structure, reproduction and evolution in selected genera*. Sunderland, MA: Sinauer Association.
- . 1979. Electron microscopy and the phylogeny of green algae and land plants. *Am. Zool.* 19:545–54.
- Pirozynski, K. A., and D. W. Malloch. 1975. The origin of land plants: A matter of mycotrophism. *BioSystems* 6:153–64.
- Pratt, L. M., T. L. Phillips, and J. M. Dennison. 1978. Evidence of non-vascular land plants from the early Silurian (Llandoveryan) of Virginia, U.S.A. *Rev. Palaeobot. Palynol.* 25:121–49.
- Pryce, R. J. 1972. The occurrence of lunularic acid and abscisic acid in plants. *Phytochem.* 11:1759–61.
- Raven, J. A. 1977. The evolution of vascular land plants in relation to supracellular transport processes. *Adv. Bot. Res.* 5:153–219.
- Remy, W. 1982. Lower Devonian gametophytes: Relation to the phylogeny of land plants. *Science* 215:1625–27.
- Rhoads, D. C., and J. W. Morse. 1971. Evolutionary and ecologic significance of oxygen-deficient marine basins. *Lethaia* 4:413–28.
- Schopf, J. W. 1970. Precambrian microorganisms and evolutionary events prior to the origin of vascular plants. *Biol. Revs. Camb. Philo. Soc.* 45:319–52.
- Schuster, R. M. 1979. The phylogeny of the Hepaticae. In *Bryophyte systematics*, ed. G. S. C. Clarke and J. G. Duckett, 41–82. London and New York: Academic Press.
- Smith, A. J. E. 1978. Cytogenetics, biosystematics and evolution in the Bryophyta. *Adv. Bot. Res.* 6:195–276.
- Stebbins, G. L., and G. J. C. Hill. 1980. Did multicellular plants invade the land? *Am. Nat.* 115:342–53.
- Stewart, K. D., and K. R. Mattox. 1975. Comparative cytology, evolution and classification of the green algae with some consideration of the origin of other organisms with chlorophylls a and b. *Bot. Rev.* 41:104–35.
- . 1978. Structural evolution in the flagellated cells of green algae and land plants. *BioSystems* 10:145–52.

- Strother, P. K., and A. Traverse. 1979. Plant microfossils from Llandoveryian and Wenlockian rocks of Pennsylvania. *Palynology* 3:1-21.
- Swain, T. W. 1980. Flavonoids. In *Pigments in plants*, ed. F.-C. Czygan, 224-36. Berlin: Springer.
- Taylor, T. N. 1982. The origin of land plants: A paleobotanical perspective. *Taxon* 31:155-77.
- Walker, D. B. 1983. The response of epidermal cells. In *Compatibility responses in plants*, ed. R. Moore, 123-37. Waco: Baylor Univ. Press.
- Walker, J. C. G., C. Klein, M. Schidlowski, J. W. Schopf, D. J. Stevenson, and M. R. Walter. 1983. Origin and environmental evolution of the Archaean-early Proterozoic earth. In *Evolution of the earth's earliest biosphere*, ed. J. W. Schopf, 260-90. Princeton: Princeton Univ. Press.
- Wray, J. L. 1977. *Calcareous algae*, Amsterdam: Elsevier-North Holland.